

5 Z buffer was transferred onto a Millipore Multiscreen Assay System (Nitrocellulose Immobilon NC), filtered, and then washed by filtering 200 μ l Z buffer. 100 μ l Z buffer with β ME and detergents was then added to each well, as was 20 μ l 4 mg/ml ONPG. Reactions were incubated at 30°C,
10 stopped with 50 μ l 1 M Na₂CO₃, filtered into a polystyrene 96-well assay plate, and OD₄₂₀ was determined for each assay well. β -galactosidase units were determined using the Miller formula (O.D. 420 X 1000) / (OD600*minutes*volume in mL). Z buffer is made by
15 dissolving the following in 1 L of water (16.1 g Na₂HPO₄·7H₂O, 5.5g NaH₂PO₄·H₂O, 0.75 g KCl and 0.246 g MgSO₄·7H₂O). Z buffer with detergents and β ME is made as follows: 9.8 ml Z buffer, 100 μ l 20 mg/ml CTAB, 100 μ l 10 mg/ml sodium deoxycholate, and 69 μ l β ME. Control plasmids utilized in
20 these studies included MB968, MB2478 and MB1644.

Results of these studies are presented in Figures 2-5, demonstrating increased transcription-activating properties of the *lovE* variants disclosed herein.

25 **Example 6: Secondary Metabolite Production**

Transformation of filamentous fungi was performed according to the following procedure. Protoplasts were generated by inoculating rich media with spores. Spores were allowed to germinate for about 20 hrs or until germ 30 tubes were between 5 and 10 spore lengths. The germlings were centrifuged and washed twice with sterile distilled water and once with 1 M magnesium sulfate. Germlings were then resuspended in 1M magnesium sulfate containing approximately 2 mg/ml of Novozyme. Tubes were then 35 incubated at 30°C shaking at 80 RPM for about 2 hrs or until most of the hyphae were digested and protoplasts were abundant. Protoplasts were filtered through one layer of Miracloth. At least one volume of STC was added and protoplasts were centrifuged. Protoplasts were washed

5 twice with STC. Protoplasts then were resuspended in 1ml STC and counted in a hemacytometer. A final concentration of approximately 5×10^7 protoplasts/ml were frozen in a 9:1:0.1 solution of STC, SPTC and DMSO in a Nalgene Cryo cooler at -80°C (cools -1°C/min).

10 Solutions for transformation were as follows: STC (0.8 M Sorbitol, 25 mM Tris-HCl pH 7.5, 25 mM CaCl₂) and SPTC (0.8 M Sorbitol, 40% PEG 4000, 25 mM Tris-HCl pH 8, 50 mM CaCl₂). Transformation was accomplished according to the following protocol. 1-5 µg of DNA comprising a *lovE* 15 variant according to the invention in a fungal expression vector was placed in a 50 ml Falcon tube. 100 µl of previously frozen protoplasts were added to the DNA, gently mixed, and then incubated on ice for 30 min. 15 µl of SPTC was added, followed by mixing by tapping and 20 incubation at RT for 15 min. 500 µl SPTC was added and mixed well by tapping and rolling, then incubated at RT for 15 min. 25 mls of regeneration minimal medium was added, mixed well and poured on plates containing 25 mls of regeneration minimal medium with 2X the concentration 25 of selection drug.

Transformation plates were incubated at 26°C for 5-6 days or until colonies started to appear. Regeneration minimal medium contains trace elements, salts, 25 mM sodium nitrate, 0.8 M Sucrose, and 1% agarose at pH 6.5.

30 The selection drug that was used successfully with *A. terreus* is phleomycin, a broad-spectrum glycopeptide antibiotic. Transformants were picked onto new plates with a toothpick (if the fungus was sporulating) or with sterile forceps (if the fungus did not sporulate). 35 Purification plates contained minimal medium (same as regeneration minimal medium but containing 2 % instead of 0.8 M sucrose) and 1X drug concentration. Picked transformants were incubated at 26°C for 5-6 days.

40 Transformants were grown in production media for secondary metabolite production. Briefly, for *A. terreus* and lovastatin production, spores were used as the inoculum. Spores were obtained from the purification

5 plate by using a wooden inoculation stick. The medium was
RPM containing corn steep liquor, sodium nitrate,
potassium phosphate, magnesium sulfate, sodium chloride,
P2000 (Dow chemical), trace elements and lactose or
glucose as carbon source. The medium was pH 6.5. Flasks
10 were incubated at 26°C with shaking at 225 RPM. For static
96-well cultures, the same medium was used and the spores
were obtained from the purification plate with a wooden
toothpick. 96-well plates were incubated, without shaking
at 26°C.

15 Sampling was done after after 5 days for
lovastatin. For shake flask experiments 1-1.5 mls of
supernatant was placed into 96-well plates, which were
centrifuged and supernatants transferred to new 96-well
plates. Samples were frozen at -80°C for storage or for
20 later assays.

Cultures that were grown standing in a 96-well plate
were centrifuged and the supernatant was transferred to a
new 96 well plate. Samples were frozen at -80°C.

25 **Example 7: Measurement of Secondary Metabolite Production**

The concentration of the secondary metabolite
lovastatin was determined by enzyme inhibition assay
(Figure 6). Briefly, 10 μ L of sample was removed and
diluted 1:100 in H₂O. 10 μ l of this diluted broth was
30 assayed in a reaction (200 μ L total) containing 1 mM
HMGCoA, 1 mM NADPH, 0.005 mM DTT and 5 μ l (His)₆HMGR. The
disappearance of absorbance at 340 nm was observed over
time. This represents the disappearance of NADPH, and
lovastatin inhibits this reaction.

35 The initial velocities were calculated for the
reactions containing samples, adjusted for dilution, and
compared to reactions containing lovastatin standards to
determine levels of metabolite produced. (His)₆HMGR was
expressed in *Saccharomyces cerevisiae* and purified with a
40 nickel column.

The results from ten individual transformants for
each allele are shown in standard box plot format in